THE ORIGIN OF THE ORGAN-FORMING MATERIALS IN THE FROG'S EMBRYO.

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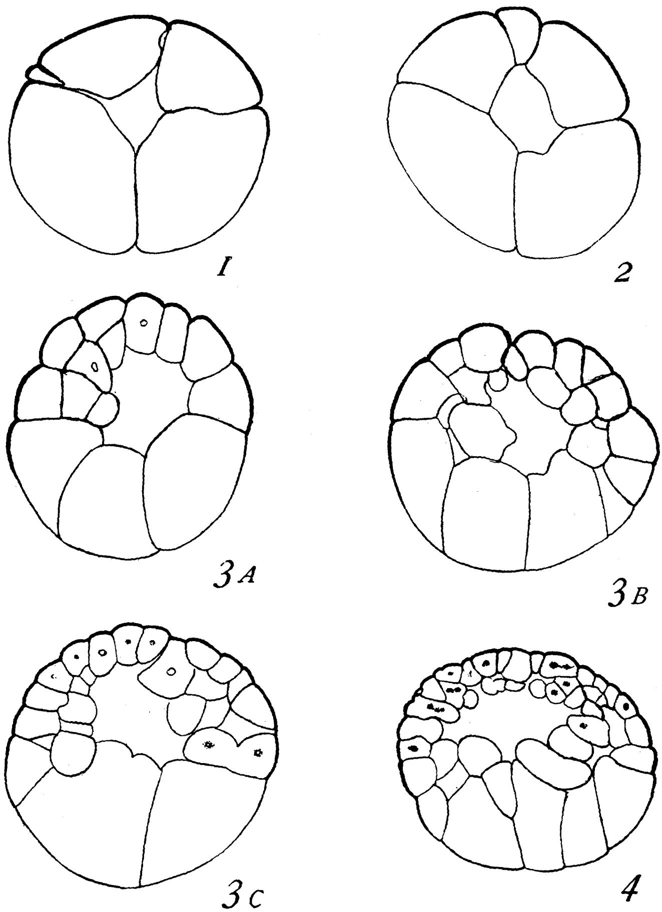
The location of the neural plate of the frog's egg, and of the organs lying immediately below it — the "embryo" in a narrow sense — has been variously determined by different embryologists. Pflüger, Roux, Schultze, O. Hertwig, Morgan, Brachet, H. V. Wilson, Assheton and others have considered this question, but since the literature has been sufficiently reviewed in recent years I shall refrain here from further comment. In the present communication I shall deal with a problem somewhat different from the location of the embryo, namely, the origin of the materials out of which the "embryo" is formed. If I can make good my point it will be seen that the embryo-forming material originates from a part of the egg different from that in which the embryo first appears, hence the location of the embyro on the egg does not give a sufficient answer to the problem of the real source of the materials out of which the embryo develops.

In a series of papers, dealing with the relation between normal and abnormal development of the frog's egg, I was led to certain conclusions in regard to the origin of the organ-forming substances, and in the last paper of the series I dealt directly with this question, basing my conclusions upon a reëxamination of normal development of *Rana palustris*. The present paper deals with the same problem in *Rana sylvatica* and *Bufo lentiginosus*, and includes some new observations on *Rana palustris*.

ARGUMENT.

Briefly stated, my view is as follows: The material, out of which the "embryo" develops, lies in the upper hemisphere, and is transported below the equator of the egg during the segmentation stages. This material forms a ring around the lateral wall of the segmentation cavity, at first above, later below the equator of the egg. The same material can be traced with some prob-

ability to the eight-cell stage, where it exists partly in the upper four cells and partly in the upper ends of the larger lower four cells. In the later segmentation stages, and in the pregastrula stages, this material moves down around the sides of the egg until it comes to lie some distance below the floor of the segmentation cavity. Here it becomes, as described above, the germ-ring, which closing over the lower hemisphere produces the "embryo."

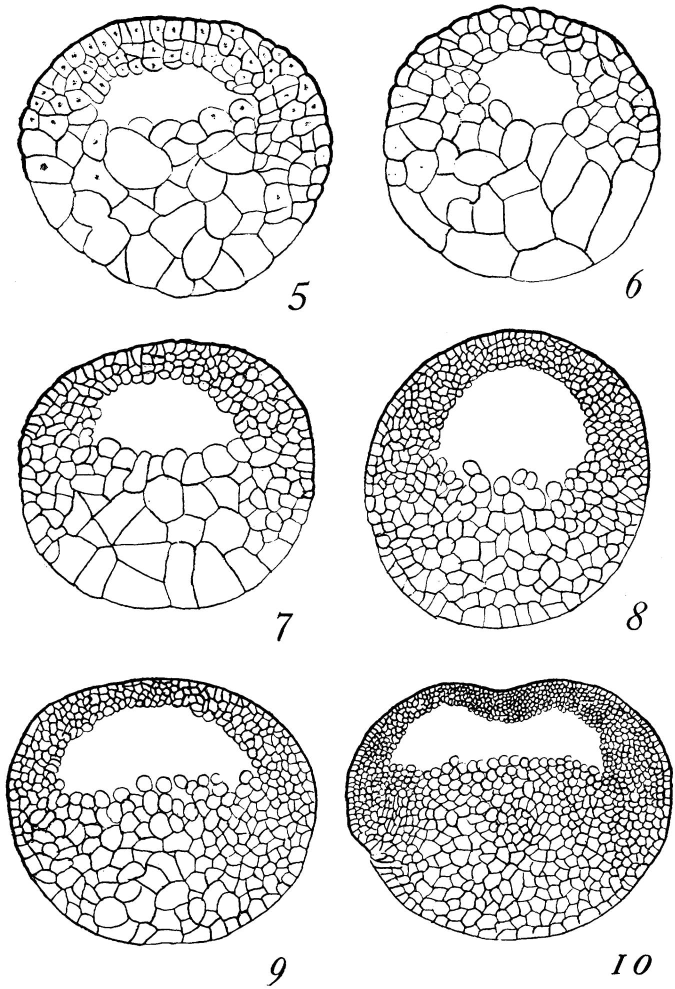


FIGS. 1–4. Segmentation stages of the *Rana palustris*.

The details of these changes, and the evidence on which these statements rest, may now be considered more fully.

DESCRIPTIVE.

Rana palustris. — Since I now have a more complete series of stages of the eggs of this species, and since I have elsewhere especially considered this form, it may be dealt with first.



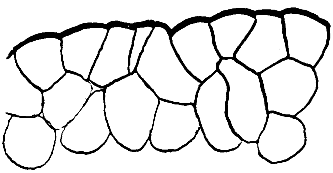
FIGS. 5-10. Later segmentation stages and gastrula stage (Fig. 10) of *Rana palustris.*

Fig. 1 is a vertical section of an eight-celled stage. The segmentation cavity is present and is bounded above by the upper four cells (of which two are seen in the section), and at the sides

by the upper ends of the lower four cells. The great thickness of the upper four cells that form the roof of the blastocœl is especially to be noted. The next section, Fig. 2, is a section through a 16-cell stage. The same relations noted for the last stage hold here also. The next three figures are from another set of eggs. Fig. 3, *A*, is a 54-cell stage with six of the cells inside. The cells of the roof have already begun to move out towards the sides, so that the middle part of the roof is much thinner than that of the sides. The next figure, Fig. 3, *B*, is from an egg containing sixty-four cells, of which fifteen are inside. It shows changes similar to those of the last figure. Fig. 3, *C*, is through an egg with 105 cells, of which twenty-six are inside. The shifting of the cells derived from the upper four cells is still more apparent. At this time the top of the roof of the segmentation cavity is much reduced in thickness, while the sides are still quite thick. In the last three figures, the segmentation cavity is very irregular in shape. At first it is deeper in a vertical plane, but in the last stage it is broadening out in a horizontal plane. The last section, Fig. 4, is from an egg belonging to the same set as Figs. 1 and 2. The following six figures, Figs. 5 to 11, belong to still another series.

The fluid in the segmentation cavity is formed by the surrounding cells, and it may be supposed that the thinning out of the roof of the blastocœl is due to loss of fluid, but as the egg as a whole slowly increases in size during this time, the loss of fluid to the blastocœl must be made good by the absorption of water from outside. During the following stages, as seen in Figs. 5–9, the roof becomes very much thinner, as shown strikingly on comparing the sections of the roof at different stages, as seen in Figs. 11–15. It will also be noticed on comparing sections of the earlier and the later segmentation stages, that the side-walls of the segmentation cavity are at all stages very thick compared with the upper wall, and also that the smaller cells of this region are being slowly carried down at the sides of the egg. They furnish the material out of which the "embryo" is later formed. In Fig. 10 the first indications of the dorsal lip are present, as seen to the left. At this stage the smaller cells have pushed down below the equator of the egg on the side at which

the dorsal lip of the blastopore lies. At the same time the lateral wall of the blastocœl has been reduced in thickness, and there can be little doubt that the reduction must be directly due to the movement downwards of its cells. On the opposite side of the section where the ventral lip will form later the down-growth of small cells is less marked, and the lateral wall of the blastocœl is correspondingly thicker. In other words, the ring of embryo-forming material lies at this time obliquely on the egg one side having pushed down further than the other. On the right and left sides of the embryo, as seen when cross-sections are made, the ring shows all intermediate stages between the condition at the dorsal and at the ventral lip.



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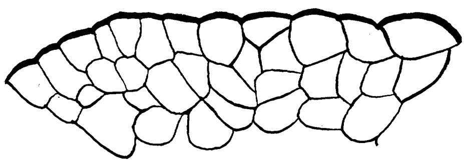

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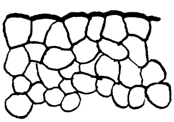

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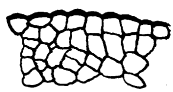

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FIGS. 11–15. Roof of segmentation cavity of *Rana palustris*.

The later history of the gastrulation has been sufficiently described in former papers. In general, the process consists of the steady migration of the embryonic ring over the lower hemisphere. As this takes place the yoke is thrown high up into the segmentation cavity, and the roof of the latter becomes reduced to a thin layer of cells.

The steady decrease in thickness of the roof of the segmentation cavity during the cleavage, pregastrula and gastrulation stages is a constant feature of the development. The decrease in thickness is well shown in the series of figures, Figs. 11–13,

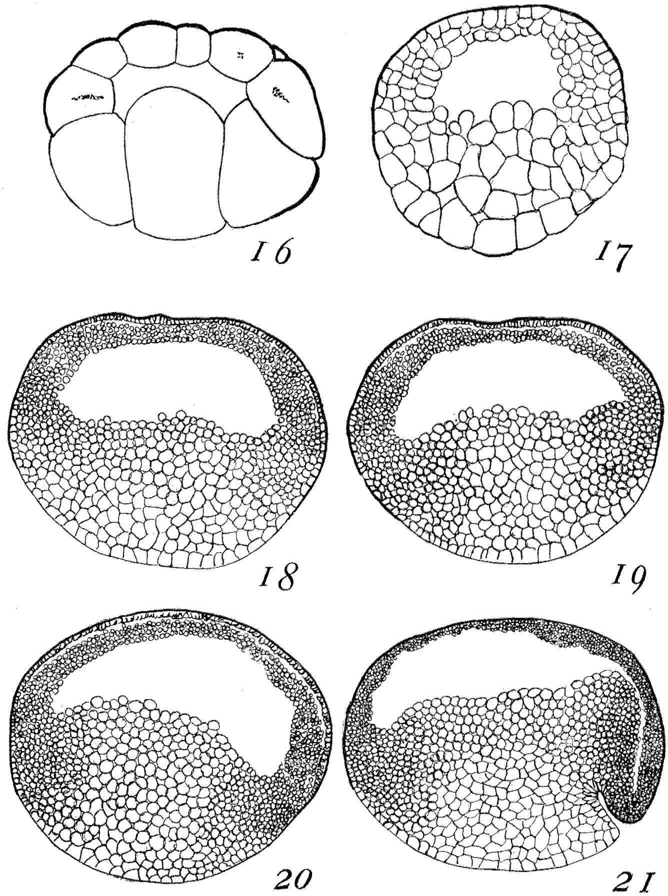
drawn to larger scale than the preceding. Since the cells are dividing during this time without the number of layers in the roof of the blastocœl becoming correspondingly greater — in fact they are fewer in the gastrulation stages — this must mean that as the cells increase in number they push out towards the sides, and increase the material of the embryo-ring, which is, *pari passu*, pushing below the equator of the egg.

Rana sylvatica. — Owing to the looseness of the cells of the roof of the segmentation cavity in this species — whether representing a real condition or due to the effects of the preserving reagents I do not know — the preparations show less well the thinning out of the roof of the blastocœl. The first stage figured, Fig. 16, is a section through a 32-cell stage. The inwandering of the upper cells has not yet been accomplished, the segmentation cavity is well developed, and its roof is thick. The upper ends of the four lower cells of the eight-cell stage are, at this time, cut off by the fifth divisions, that usually lies obliquely. In the figure these cells are represented by the two large cells at the sides. They contribute, I think, much of the material that forms the "embryo."

The next figure, Fig. 17, shows a later stage, cut somewhat obliquely. The blastocœl is large, and its roof thinner, but the sides are as thick as before, or nearly so, as shown by superposing the 32-cell stage upon this one. The next two figures, Figs. 18–19, show a broadening of the blastocœl, and thinning of its roof, accompanied by a downgrowth of the small cells around its sides. The next figure, Fig. 20, shows the downgrowth carried further, and the yolk floor correspondingly lifted up. The last figure, Fig. 21, is through the dorsal lip of the blastopore, at the time when the invagination has just appeared. The roof of the segmentation cavity is thinner and continuous at the anterior end with the ectoderm in front of the dorsal lip. This ectoderm is now separated from the cells inside; except in the dorsal lip itself, where ectoderm and mesoderm completely coalesce.[1] A tongue of small cells, running upwards from the blastopore beneath the ectoderm, represents the mesoderm. This mass of cells along with the yolk-cells

[1] Possibly ectoderm cells are added along the middle line to the sheet of mesoderm and give rise to the notochord.

with which it is continuous further inwards, projects upwards at the anterior end of the egg into the blastocœl. On the opposite side of the section, the downgrowth is less developed, and the yolk-floor less elevated.

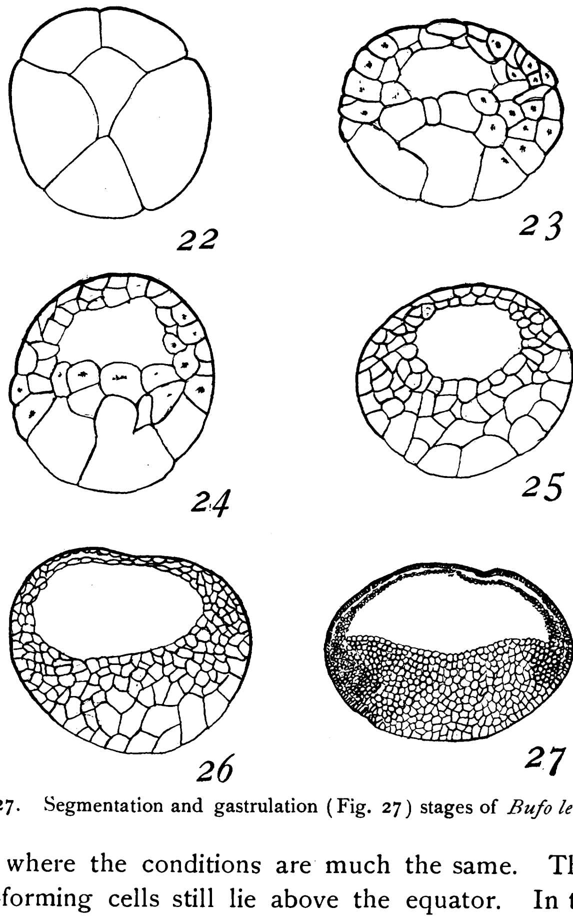


FIGS. 16–21. Segmentation and gastrulation stage (Fig. 21) of *Rana sylvatica*.

The general conditions in this species are the same, in all essential respects, as in the last.

Bufo lentiginosus. — The toad, although belonging to another family of the Anura, follows very closely the same method of early development as the frog. In several respects it shows with great clearness some of the early changes that I have described

in the frog's egg. A section through an eight-cell stage is shown in Fig. 22. The thickness of the roof and the sides of the blastocœl, and the extension of the blastocœl far down into the lower hemisphere is very noticeable. In the next stage, Fig. 23, the roof is thinner, and the segmentation cavity extends out more horizontally. The same changes are seen in the next figure, Fig. 24, which is an egg in the same stage of development, but compressed, in cutting, in a plane at right angles to that of the last figure. An older stage is seen in the next figure,



FIGS. 22–27. Segmentation and gastrulation (Fig. 27) stages of *Bufo lentiginosus*.

Fig. 25, where the conditions are much the same. The small embryo-forming cells still lie above the equator. In the next stage, Fig. 26, the segmentation cavity is greatly enlarged, its roof is much thinner, and now the embryo-forming cells lie at

about the equator of the egg. It is evident, when this stage is compared with the last one, that an extensive shifting of the cells must have taken place. This is strikingly shown by superposing the outline of Fig. 22 upon that of Fig. 26. The next stage, Fig. 27, shows important changes to have taken place in the small cells around the sides of the segmentation cavity. These have now pushed below the equator of the egg, and the wall in this region has correspondingly diminished in thickness. This last section is through an embryo that has just reached the gastrulation stage, and although the section passes vertically through the greater diameter of the egg, the blastopore lip lies slightly to one side, and opens in neighboring sections that are not quite so large. The mesoderm cells, containing more pigment than the endoderm cells, form a distinct tongue extending upwards from the level of the blastopore to the floor of the archenteron. The general appearance of the section suggests strongly that the innermost cells have remained stationary, or have even pushed upwards a little, accompanying the general upward movement of the floor of the archenteron.

The later stages of the gastrulation-process in the toad, I have not studied. King has recently given a series of figures showing the later development. They show that the yolk-mass is thrown high up into the blastocoel, especially at the anterior end, so that the anterior part of the archenteron comes to lie higher up than the original floor of the archenteron. It is not improbable that the embryo of the toad lies higher up on the anterior side of the egg than does the embryo of the frog, although the difference is only one of degree.

During the period of closure of the blastopore, the large blastocœl becomes filled by the yolk and the drawing inwards of the yolk brings the dorsal and ventral lips together without, in a sense, their actually growing *over* the lower hemisphere.

Conclusions.

The large number of cells in the frog's egg at the time when the embryo appears makes it impossible to trace the cell-lineage after five or six divisions, so that we must be content with less refined methods in locating the embryo-forming materials.

Whether the inwandering, or inpulling of some of the cells of the upper hemisphere is only due to the shifting of the cells as they divide, so that the pressure relations are better adjusted, or whether there is a further meaning to be attached to this process cannot be stated. One might be tempted to assign to the cells the function of producing the mesoderm, and the later pushing outwards at the sides of much of the material derived from these cells into a position where the mesoderm appears might be made to give color to this interpretation. However this may be, the experiment of removing the upper four cells shows that some, at least, of the mesoderm comes from other parts of the egg. Whether the same amount forms under these circumstances is too difficult to determine. In the later stages of gastrulation, and at the time also when the neural plate is forming, the mesoderm on the ventral surface increases at the expense of the yolk-cells. These are the yolk-cells that form the tongue of cells that pushes upward into the blastocœl.

In my last paper dealing with the gastrulation of the frog's egg, I discussed the "mechanics" of the downward movement of the material that forms the embryonic ring. Certain points that bear on this question have been noted in studying the three species here described, and may be briefly mentioned. There seems to be a good deal of variation in the extent to which the blastocœl enlarges, not only in different species, but in the same species when eggs from different bunches are compared, and even to a slight extent in eggs from the same bunch. The segmentation cavity appears to be formed as a result of the secretion of material from the surrounding cells, as shown by its occurrence in eggs developing out of water. The egg as a whole increases in size as the segmentation cavity grows larger, indicating that the water absorbed from outside more than compensates for the amount of fluid secreted into the central space.

The thinning of the roof of the segmentation cavity takes place at the time of enlargement of the segmentation cavity and it may appear that this enlargement is the cause of the downward movement of the material; but that this is not the correct interpretation of the mechanics of the process is shown by the following experiment. The top of the segmentation cavity was

opened and partly removed, by means of a needle, at different stages in the cleavage process. Nevertheless, the materials that form the embryonic ring continued to push down at the sides of the egg and a normal embryo was produced. The experiment is open to the obvious criticism that after the removal of the roof, or a part of it, the opening soon closes again; but if the downgrowth were really due to pressure of the blastocœl fluid or even to the pushing of the cells of the roof on each other, the disorganization of the process, that would probably follow, when a part of the roof is removed, must be so great, one would think, that it is unlikely the downgrowth could subsequently take place normally. Still the experiment is inconclusive and does not settle the question.

The two most conspicuous changes that take place during the segmentation and gastrulation stages are the development of the enormous segmentation cavity and its disappearance during gastrulation. We may look upon the *purpose* of the segmentation cavity as a space into which the lower cells may push when the upper cells pass over them. While the presence of the segmentation cavity may facilitate this process, we gain no insight into the origin of the cavity or of its disappearance by a consideration of its purpose. Its formation seems to be due largely, as I have already said, to a secretion of fluid from the surrounding cells; its disappearance is more difficult to explain. The question resolves itself into these alternatives — is the blastocœl fluid absorbed or is it forced out of the egg in the later stages of gastrulation? Since the egg does not decrease in volume or very little, as the yolk surrounds and finally obliterates the segmentation cavity, the fluid would seem to be absorbed. It is true, we might assume that the fluid is squeezed out of the egg and water from the outside absorbed at the same rate, but this assumption only complicates the question, and in the end amounts to the same thing. Admitting that the fluid is absorbed, can this explain the inward migration of the yolk. I think not, because in the first place the process of ingrowth takes place around the sides of the blastocœl cavity and around the lips of the blastopore and not throughout the entire floor of the segmentation cavity, and in the second place there is nothing in the changes that take place

to indicate that they are due only to the absorption of the blastocœl fluid, since the cells that undergo the changes in question do not appreciably grow larger than the others. On the contrary the gastrulation seems to be due to the change in shape and migration of certain cells, and the absorption of the fluid from the blastocœl appears to be no more than the ordinary process of water absorption that takes place throughout the whole period of development.

The evidence that we have at present seems to indicate that the process of gastrulation is due to the activities of the cells themselves, *i. e.*, the "mechanics" of the process can only be explained by an appeal to the response of the cells to certain stimuli. The actual changes that we observe involve a change in shape of certain cells, and our problem resolves itself into determining what stimulus leads to this change in shape, and what physical process is involved in the change. I have pointed out that it seems to me probable that the stimulus is derived from the mutual pressures of the cells, and the change of shape is due to contraction processes in the cells (that lead to their change in shape) that are akin to the same contraction shown by a protozoön, or, in a higher form, by muscle cells. The different behavior of the cells in different parts of the egg must be ascribed to their difference in materials that are derived from the different parts of the egg. After each cell-division, itself apparently a process of local contraction, there must be a rearrangement of the pressure relations in the different parts of the egg.

The formative factors of development can be reduced, from this point of view, to the two generally recognized properties of living matter, irritability and contractility. The stimulus that arouses the irritability is the pressure relation of the cells; the time at which each cell is effected by this stimulus will depend on its material composition, and it responds by its contraction. If this interpretation of the formative changes is correct it refers the process of development to two physiological properties of living matter, and not directly to other known physical properties of organic matter. What the physical bases of irritability and of contractility are remains for the future to decide. They may be

simply complexes of known physical factors, or they may be physical properties of organic matter that are not met with in inorganic compounds. Whatever their ultimate nature it would be a distinct gain if we could prove that most of the phenomena of early development, the so-called formative changes, are due to these two properties of living substance.